NanoEnTek

CE IVD

FREND™ Testosterone

Total Testosterone

Intended use

The FREND™ Testosterone test is a fluorescent nanoparticle immunoassay designed for *in vitro* quantitative measurement of total testosterone in human serum and plasma (EDTA and Lithium-heparin). The FREND™ Testosterone microfluidic flow cartridge is designed for use in the FREND™ System fluorescent immunoassay reader.

Summary and explanation of test

Testosterone is a male sex hormone, secreted by Leydig or interstitial cells of the testes. Testosterone levels are controlled by luteinizing hormone, secreted by the pituitary gland, via negative feedback on the pituitary gland and hypothalamus. Serum concentrations of testosterone sequentially rise and fall through the fetal stage until 6 months after delivery due to maternal hormonal changes, and then remain low (e.g., 0.3 ng/mL) between six months of age and puberty. After puberty the increase of testosterone levels in males is gradual until it reaches adult level (Table 1). In females, testosterone is produced mainly by conversion of prehormones.

Table 1 Typical Physiological Testosterone Level[1]

	Male (ng/mL)	Female (ng/mL)
Prepubertal	0.01~1.77	0.01~0.20
Pubertal	0.02~8.00	0.02~0.40
Adult	2.80~11.00	0.15~0.70

Circulating testosterone is 98% protein-bound in males, with slightly less being bound in females. The proteins responsible for binding testosterone are as Sex Hormone Binding Globulin (SHBG), also referred to as Testosterone Binding Globulin (TeBG) and serum albumin.[2]

Clinically, testosterone monitoring is used to help diagnose and differentiate endocrine disorders. In males, these include hypogonadism, testicular failure, infertility, hypopituitarism and hyperprolactinemia. In females, changes in serum testosterone levels can be caused by polycystic ovary syndrome, adrenal hyperplasia, infertility, hirsutism, amenorrhea, obesity and virilization.

Principle of the assay

The FREND™ Testosterone test cartridge is a one-time-use rapid "competitive" immunoassay utilizing fluorescent nanoparticle in microfluidic flow to capture and quantify total testosterone in serum and plasma specimens. The drop of 70µL patient sample is placed in the FREND™ Testosterone pretreatment tube, where the sample interacts with a proprietary mix of pretreatment solution. Initially, patient sample is mixed with testosterone antibody-labeled particles, forming immune complexes with total testosterone in the patient sample, then, incubated for 5 minutes at 37℃. The drop of 35 µL mixture is added to the FREND™ Testosterone cartridge, interacting with testosterone conjugated fluorescent nanoparticles. The mixture moves via Capillary action to the detection region, where fluorescent nanoparticle complexes are grabbed. The fluorescence intensities from the complexes are measured and total testosterone concentration is calculated by the FREND™ System.

Material provided

Contents		Catalogue number
20	FREND™ Testosterone cartridge(s)	FRTE 020
20	FREND™ Testosterone pretreatment tube(s)	
01	FREND™ Testosterone Code chip	
01	FREND™ Testosterone Package Insert	

One cartridge contains:

Testosterone	218.80±21.88 ng
Monoclonal anti-testosterone antibody	32.0±3.2 ng
Fluorescent particles	1.80±0.18 μg

One pretreatment tube contains:

Gold antibody conjugates	30.0±3.0 μg

Materials required but not provided

The FREND™ System Micro-pipette capable of delivering 35 and 70 μL
Heating block for tube and cartridge incubation at 37℃.
Timer for incubation step
Personal protective equipment and biohazard waste

Warning and Precautions

- The FREND™ Testosterone cartridges are intended for in vitro diagnostic use only.
- Testosterone cartridges are only to be used on the FREND™ System.
- Testosterone cartridges are disposable, single use devices. Do not reuse them under any circumstances.
- Allow sealed cartridges to come to room temperature for 15~30 minutes prior to use.
- Cartridges and pretreatment tube should not be frozen.
- Assure the humidity in the laboratory is in the 10~80% range when tests are run.
- Assure the room temperature remains in the range of 22~30 ℃ when tests are run.
- Avoid cross-contamination between samples by using a new pipette tip for each new specimen.
- Avoid high humidity, direct sunlight or heat in the area used for cartridge storage.
- Inaccurate results are possible if the sample used is contaminated in any way.
- Using specimens containing clotted fibrin could result in erroneous results.

- Over or under loading the cartridge with sample may result in inaccurate results.
- Human specimens are not used in the preparation of this product, however, since human specimens will be used for samples and other quality control products in the lab may be derived from human materials. Please use Universal Precautions when handling all specimens and controls.
- Do not use the cartridges and pretreatment tubes beyond the expiration date on the pouch.
- Do not use the cartridge and pretreatment tubes if the pouch is damaged or the seal is broken.
- Perform testing as specified in the Package Insert and User Manual.
- Keep the cartridge and pretreatment tube sealed in the pouch until just ready for use.
- Use the cartridge and pretreatment tube immediately after opening the pouch.
- Wear disposable gloves when handling the cartridges, pretreatment tubes and the samples.
- Wash hands thoroughly and often after handling reagent cartridges or samples.
- Do not ingest the silica gel package found in the cartridge pouch.
- Testosterone has been designed so that the high dose "hook effect" does not affect the vast majority of samples.
- Handle specimens in accordance with the OSHA Standard on Bloodborne Pathogens.

Storage and Stability

All unopened materials are stable until the expiration date on the label when stored at the specified temperature. Reagent stability has been demonstrated for twelve months from the date of manufacture.

The expiration date is clearly indicated on the product box and the cartridges.

Materials	**Catalogue number**
Refrigerator temperature storage (2~8 ℃)	
FREND™ Testosterone cartridges	FRTE 020
FREND™ Testosterone pretreatment tubes	None

Specimen collection and handling

Human serum and plasma (EDTA and Lithium-heparin) samples are suitable for use with FREND™ Testosterone cartridges.

Follow instructions detailed in this package insert as well as the specimen collection tube manufacturer's instructions for specimen collection and preparation (including manufacturer's instructions for centrifugation time and speed.)
For serum, a blood sample is collected aseptically without additives by venous puncture. After allowing the sample to clot for 30 minutes at room temperature, the collection tube should be centrifuged for 10 minutes at 3,000 rpm.
For plasma (EDTA and Lithium-heparin), a venous blood sample is collected aseptically with the designated additive. After allowing the specimen to sufficiently mix with anticoagulant at room temperature, the sample tube can be centrifuged for 10 minutes at 3,000 rpm.
Samples may be stored at 2~8 ℃ for up to 6 hours prior to analysis. If the analysis is scheduled to be done at some later time, the sample should be stored frozen at -20℃ or below for future use.
Repeated freeze-thaw cycles should be avoided. Prior to assay, slowly bring frozen samples to room temperature (18~25 ℃) and mix gently but thoroughly before test.
For optimal results, avoid grossly hemolytic, lipemic, or turbid specimens. Specimens should be free of aggregated fibrin, red blood cells, or other particulate matter.
When pipetting into the FREND™ Testosterone cartridge sample inlet, ensure that bubbles in the sample are avoided. Bubbles may restrict flow and result in an incomplete or erroneous test result.

Procedure

Calibration

There is no need for calibration to be performed by the end user as is generally required on other automated laboratory equipment.

All calibration statistics and information have been electronically stored on the FREND™ Testosterone Code chip included in each box of FREND™ Testosterone cartridges. The FREND™ Testosterone Code chip is specific for each manufactured lot of FREND™ Testosterone cartridges.

Calibration information should always be checked by running external quality control samples to verify that the results obtained for Testosterone on the FREND™ System using the FREND™ Testosterone cartridges of a specific lot meet the laboratory criterion for acceptability.

Code chip installation

Please refer to the FREND™ System user manual for more detailed instructions relative to the Code chip installation. Abbreviated instructions follow here:

1) Insert the FREND™ System electrical cord into an appropriate outlet.
2) Insert the Code chip into the Code chip slot at the rear of the FREND™ System following the arrows.
3) Press the 'Setup' button on the 'Main' screen.
4) Press the 'Code chip' button on the 'Setup' screen.
5) The information embedded on the FREND™ Testosterone Code chip is automatically saved on the FREND™ System.
6) When the Code chip installation is completed, press the 'OK' button to go to the 'Setup' screen.
7) Press the 'Item' button on the 'Setup' screen.
8) Check the FREND™ Testosterone cartridge lot number and the installation date of the Code chip.
9) Press the 'Home' button to go to the 'Main' screen to begin running external quality control and patient samples.

Quality control

FREND™ QC Cartridge contains multiple controls to check optic part of the system. By testing QC Cartridge, part of analytical components of the system of (1) laser power, (2) alignment, and (3) mechanical integrity are confirmed.

For each day of patient testing perform QC Cartridge testing. Refer to the quality control procedures section in the User Manual of FREND™ System. In brief, perform QC Cartridge testing for the following conditions:
(1) Upon initial setup of the system,
(2) Each day of patient testing,
(3) When the system has been transported or moved,
(4) Whenever there is uncertainty about the performance of the system,
(5) Whenever required by your laboratory's quality control requirements.

• Internal procedural controls
FREND™ Testosterone test cartridge contains built-in control feature. Fluorescence signal in the reference zone of each cartridge shows: (1) that enough volume is added, (2) that proper flow is obtained, and (3) that the antibody is reactive. If this reference zone signal is missing or lower than threshold, the FREND™ System consider it as an incorrect or failed test, not producing a test result but an error message. In addition, with each cartridge run, the system monitors, in part, for (1) flow of sample, (2) speed of sample flow, (3) shelf-life of cartridge components, (4) function of internal barcode scanner, and (5) function of scanner's mechanical components.

• External quality control testing
Commercially available controls from a variety of manufacturers are available that contain 25-hydroxy Testosterone as a measured analyte. It is recommended that a minimum of two (2) levels of controls be run at least once per month or once for each new lot, whichever comes earlier. However, Controls should be run with a minimum frequency, depending on number of tests run in the laboratory. Each laboratory should establish its own criteria based on the following parameters:
(1) Each new lot,
(2) Each new shipment (even if from the same lot previously received),
(3) Each new operator (an individual who has not run the tests for at least two weeks),
(4) Monthly, as a continued check on storage conditions,
(5) Whenever problems (storage, operator, or other) are identified,
(6) Or other times as required by your laboratory's standard QC procedures.

Individual laboratory policy will dictate exactly which control materials and lot numbers should be run, the frequency with which controls are to be tested, criteria for acceptance of the results and required corrective action to be taken if results do not meet laboratory criteria. If any external quality control sample values are out of the acceptable range, it will be necessary to investigate the problem before reporting patient results to assure there is not an instrument or software malfunction. Do not assay patient samples on the FREND™ System using FREND™ Testosterone if quality control on how to determine acceptability of external control material results. Each laboratory operates under a different set of regulations. Every laboratory must follow the standardized procedures acceptable to the regulatory agencies to which the laboratory is responsible.

Specimen processing

• Preparation

Remove sufficient cartridges and pretreatment tubes of FREND™ Testosterone from the refrigerator to test the number of patient samples and required external quality materials. Allow the tubes and the sealed pouches containing the cartridges to come to room temperature for 15~30 minutes prior to the start of the testing sequence. Heating block provided with FREND™ System should be turned on 7~8 minutes before use.

If using refrigerated patient samples, remove those from the refrigerator and allow to them to come to room temperature prior to testing. If frozen samples will be utilized, be sure these are removed from the freezer, thawed naturally and then mixed gently but thoroughly prior to testing. Testing should not begin on these previously frozen samples until they have reached room temperature.

There are no other reagents or sample preparations necessary.

• Assay procedure

1) Prepare the FREND™ Testosterone cartridge, pretreatment tube and specimen. Open the pouch and place the FREND™ Testosterone cartridge on the heating block at 37 ℃.
2) Transfer 70 µL of specimen to the pretreatment tube and mix well.

⚠ Caution: Pellet in the tube bottom should be completely dissolved.

3) Put the tube in the hole of heating block and incubate for 5 minutes at 37 ℃.
4) Pipette 35 µL of the incubated sample into the sample inlet on the cartridge using a suitable micro-pipette equipped with a fresh pipette tip.
5) Press the 'Test' button on the 'Main' screen of the FREND™ System.
6) The system moves to the Patient ID screen automatically.
7) Type the Patient ID and press the 'Enter' button to begin the test.
8) Insert the cartridge into the cartridge slot using the cartridge arrow as a guide.

⚠Caution: Please check the direction of the cartridge before insertion and assure the insertion is complete. It is recommended to insert the cartridge after the sample loading after 30 seconds elapsed in less than 5 minutes to obtain the optimal result of test.

9) When the reaction in the cartridge is completed, the FREND™ System will automatically begin the reading process.
10) When the measurements are completed, the cartridge will automatically be expelled and the results displayed.

⚠Caution: Do not disconnect power cord or shut off power from the FREND™ System while a cartridge is in the reading chamber. This may cause a system error.

11) If the FREND™ System is connected to the optional printer, press the 'Print' button and the results will be output on the printer paper.
12) For more detailed instructions, please refer to the FREND™ System User Manual.

Procedural notes

If a specimen Testosterone concentration is found to be greater than the linearity limit of the assay of 15.00 ng/mL and a definitive result is required, the specimen should be diluted with low concentration sample that has been previously measured on the FREND™ Testosterone and then re-assayed according to the 'Assay Procedure'. The recommended dilution for samples with an initial result of >15.00 ng/mL is 1:2. Dilutions must be made manually and the final result on the diluted sample calculated manually by multiplying the result obtained on the diluted sample by the dilution factor.

※ Original Unknown Concentration of Testosterone = Concentration of diluted sample * 2 – Concentration of low level sample

Calculation of results

The FREND™ System performs all sample and reagent handling operations automatically within the cartridge once the sample has been manually loaded to the sample inlet in the cartridge and the cartridge placed into the FREND™ System. The rate of fluorescence produced by the reaction is read at various intervals during the analysis process, blank reading are subtracted after which the net rate is automatically converted to Testosterone concentration in ng/mL based upon information stored on the FREND™ Testosterone Code chip.
This result is then output on the screen and to the optional printer. It is also stored in memory on the FREND™ System.

Screen displayed for various concentration scenarios

Displayed result	Description
	Testosterone concentration Less than 0.20 ng/mL
	Testosterone concentration Not less than 0.20 ng/mL and not higher than 15.00 ng/mL
	Testosterone concentration Higher than 15.00 ng/mL

Limitations of the procedure

1) When used for diagnostic purposes, the results obtained from this assay should be used in conjunction with other data (e.g, symptoms, results of other tests, clinical impressions, medical history, therapy, etc.)
2) The FREND™ System paired with a FREND™ Testosterone cartridge, is programmed to report 15.00 ng/mL as the highest concentration of Testosterone measurable without dilution. The lowest measurable concentration is 0.20 ng/mL – the assay limit of detection.
3) Specimens from patients with heterophilic antibodies, such as anti-mouse (HAMA), anti-goat (HAGA), or anti-rabbit (HARA) antibodies, maybe show falsely elevated or depressed values or may result in an incomplete test. Patients routinely exposed to animals or animal serum products can be prone to these types of heterophilic interferences.
4) Certain medications may interfere with assay performance. All results should be interpreted with respect to the clinical picture of the patient.
5) Although hemolysis has an insignificant effect on the assay, hemolyzed samples may indicate mistreatment of a specimen prior to assay and results should be interpreted with caution.
6) Lipemia has an insignificant effect on the assay except in the case of gross lipemia where interference with the lateral flow of the sample in the cartridge may occur.
7) The concentration of Testosterone in a given sample determined with assays from different manufacturers can vary due to differences in assay methods, calibration, and antibody specificity.
8) Please refer to the Specimen Collection and Handling, Warnings and Precautions, Storage and Stability, and Procedural Notes sections in this insert sheet.
9) FREND™ Testosterone has not been validated in point-of-care settings.
10) FREND™ Testosterone is to be used in licensed clinical laboratories with trained technologies.

Performance characteristics

Performance characteristics were evaluated for the FREND™ Testosterone as follows:

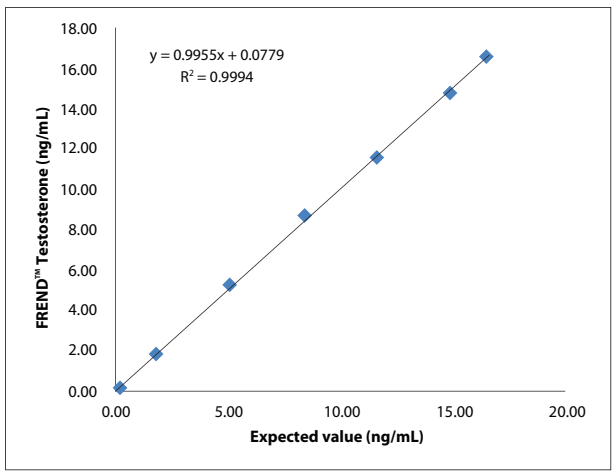
Precision

Clinical and Laboratory Standards Institute (CLSI) document EP5-A2 was utilized as a guidance for precision studies on FREND™ Testosterone. Three human serum based panels were assayed in two replicates, twice per day over a period of 20 days.

sample ID	Mean (ng/mL)	Within-run		Between-run		Between-day		Within-laboratory	
		SD	CV%	SD	CV%	SD	CV%	SD	CV%
1	1.56	0.128	8.2	0.047	3.1	0.010	0.6	0.137	8.8
2	5.98	0.313	5.2	0.054	0.9	0.135	2.3	0.345	5.8
3	12.04	0.609	5.1	0.239	2.0	0.094	0.8	0.660	5.5

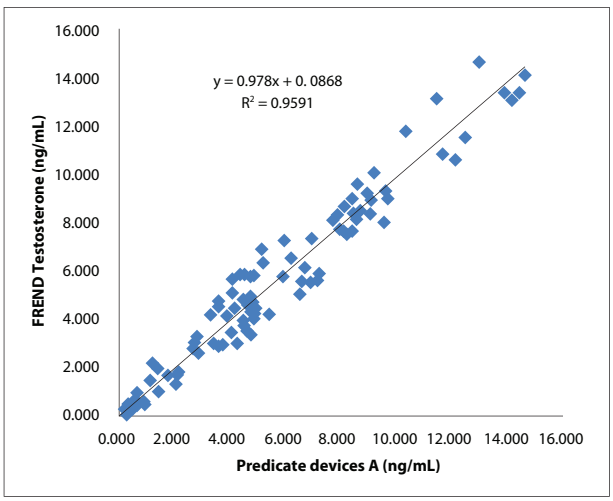
Dilution linearity

The range of linearity in serum was established using CLSI document EP6-A. FREND™ Testosterone was found to have linearity within the reportable range of 0.20 ng/mL ~ 15.00 ng/mL with a mean recovery of 100±10%. The dilution linearity study was performed by diluting a high concentration Testosterone specimen with a low concentration Testosterone specimen. Results are summarized in the graphs below, but may vary in individual laboratories.



Comparative analysis

FREND™ Testosterone was compared to the predicate device using guidelines outlined in CLSI document EP09-A2-IR. Samples (n=120) were measured in duplicate on both systems. Linear regression analysis was utilized to demonstrate a slope that 1.0 (±0.2) and a correlation coefficient (R^2) of ≥0.92.



Specificity

FREND™ Testosterone demonstrates cross-reactivity(%) with the following related substances at the concentrations indicated bellows. CLSI document EP7-A2 was used to analyze and calculate cross-reactivity using serum samples at three levels of Testosterone within the normal range.

Substance	Concentration	Testosterone concentration level		
		Low	Median	High
		Cross reactivity (%)		
17-α-ethynylestradiol	100 ng/mL	99.8	97.0	98.5
17-α-hydroxyprogesterone	100 ng/mL	106.8	103.9	96.0
19-Norethinrone	100 ng/mL	101.5	93.2	102.7
19-Norethindrone acetate	100 ng/mL	94.4	93.1	93.4
Cholramphenicol4-Androsen-3, 17-dione (Androstenedione)	10,00 ng/mL	108.5	99.3	98.4
5-α-DHT	50 ng/mL	97.0	100.3	105.7
Androsterone	50,000 ng/mL	100.9	106.1	105.5
Corticosterone	5,000 ng/mL	105.0	99.8	101.8
Cortisone	1,000 ng/mL	99.8	96.9	103.5
Danazol	1,000 ng/mL	105.6	105.8	96.9
Estradiol	1,000 ng/mL	89.1	98.9	96.7
Estriol	100 ng/mL	93.9	98.6	97.4
Estrone	500 ng/mL	94.7	95.4	106.1
Norgestrel	100 ng/mL	90.4	94.1	105.8
Progesterone	1,000 ng/mL	97.2	98.2	95.1
Reichstein's substance S (11-Deoxycortisol)	1,000 ng/mL	94.4	92.8	100.7
Trans-Dehydroandrosterone (DHEA)	10,000 ng/mL	106.8	107.3	103.2

Analytical sensitivity

The limit of blank (LoB) and limit of detection (LoD) was determined using guidelines found in CLSI document EP17-A. LoB was determined from 60 replicate measurements using a calibrator A(Testosterone depleted serum). LoD was determined using 12 replicates measurements of five low level patient samples.

LoB (ng/mL)	LoD (ng/mL)	LoQ (ng/mL)
0.10	0.14	0.14

Interference

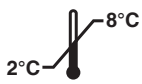
FREND™ Testosterone demonstrates ≤10% interference with the following substances at the concentrations indicated below: Hemoglobin, Bilirubin, Triglycerides, Total protein and Biotin. CLSI document EP7-A2 was used to analyze and calculate percent interference using serum samples at three levels of Testosterone within the normal range.

Substance	Concentration
Hemoglobin	100 mg/dL
Bilirubin	15 mg/mL
Triglyceride	1 g/dL
Total protein	12 g/dL
Biotin	30 g/dL

References

1) Burtis C.A. and AshwoodE.r., Ed. Tietz Textbook of Clinical Chemistry, 2nd Edition, W.B. Saunders Company. 1994, 1850-51.
2) Dunn J.F. , Nisula B.C. and Rodbard D. *Journal of Clinical Endocrinology and Metabolism*, 1981, 53: 58-68.

Glossary of symbols

	Do not reuse
	Use by YYYY-MM-DD
	Lot number
	Catalog number
	Warning or Caution
	Manufactured by
	Authorized representative in the Europe Community
	In vitro diagnostic medical device
	Temperature limitation
	Contains sufficient for <n> tests

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